

SEROPREVALENCE OF *Neospora caninum* IN CATTLE OF NEISHABOUR, NORTHEAST IRAN

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Abstract: *Neospora caninum* is a worldwide distributed pathogen which causes abortion in cattle and leading to economic loss in the cattle industry. The aim of this study was to determine the seroprevalence of *N. caninum* antibodies in cattle in Neishabour (Northeast of Iran). From September 2012 to October 2013 a total of 100 serum samples were analyzed for antibodies against *N. caninum* antigen using a commercial *N. caninum* ISCOM ELISA kit. Antibodies to *N. caninum* were found in 26 of the 100 (26%) sera based on ELISA test results. With regard to seropositivity, no significant difference was observed regarding origin, sex and age ($P>0.05$). The results showed that *N. caninum* is relatively prevalent in cattle in the Northeast part of Iran and the evaluation of potential of infection can be useful when considering control programs.

Key words: seroprevalence; *Neospora caninum*; Neishabour; cattle; Iran

Introduction

Neospora caninum is a heteroxenous cyst-forming apicomplexan protozoan which is considered as a major causative of infectious bovine abortion worldwide and has been associated with sporadic, endemic and epidemic abortions (1, 2). The infection causes important economic loss to the cattle industry due to reproductive failure associated with abortion and mortality in congenitally infected calves. *N. caninum* infection

has been reported in dairy cattle herds on all continents (3, 4, 5, 6).

Dogs and coyotes are the definitive hosts in *N. caninum* life cycle (7, 8, 9) whereas cattle and other mammals act as natural intermediate hosts (10, 11, 12). In cattle, *N. caninum* infection may occur by horizontal transmission due to ingestion of sporulated oocysts shed by the definitive host (13, 14). However, vertical transmission is the predominant route of infection (1, 14). Vertical transmission occurs when tachyzoites cross the placenta of a persistently infected dam and infect the fetus (15).

Transplacental transmission can occur in consecutive pregnancies in the same cattle and so the infection can persist in cattle herds through many generations. The infection usually has a chronic course and persists throughout the life of an infected animal (16, 17). *N. caninum* DNA has been reported in fresh and frozen semen of naturally infected bulls and the possibility of venereal transmission in bovine neosporosis has been suggested (16, 18).

The economic impact of *Neospora*-induced abortions depends on direct costs and the value of fetuses lost. Indirect costs include those associated with establishing the diagnosis, rebreeding cows that aborted and possible loss of milk yield (19). As clinical diagnosis is difficult, serological tests are necessary for an exact diagnosis. Several serological tests, including the enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody technique (IFAT), the direct agglutination test (DAT), and immunoblots (IB) can be used to detect anti *N. caninum* antibodies (13).

Iran is a very diverse country in terms of climate. To evaluate the impact of climate on the prevalence of *N. caninum*, the incidence of this parasite should be measured in different climatic areas. Since recognition of *N. caninum* in 1980, there are only few studies about the seroprevalence of bovine neosporosis in Iran, which are mostly carried out in Central and South-East Iran (20, 3). This study was performed to determine the prevalence of antibodies to *N. caninum* in cattle in Neishabour city, Northeast of Iran.

Materials and methods

Serum samples

During September 2012 and October 2013, One hundred serum samples were collected from cattle of Neishabour, the animals being randomly selected. Blood samples were taken using disposable needles. The owners were questioned about animal management, age and the information obtained was recorded. This study was performed between September 2012 and October 2013. All samples were immediately transported to the diagnostic laboratory. Serum was removed after centrifugation at 1000×g for 10 min. All sera were divided equally into two

microtubes and stored at -20 °C until laboratory testing.

ELISA test

Serum samples were stored at -20°C until tested. They were analyzed for antibodies to *N. caninum* using ELISA. Anti-Neospora antibodies were detected using a commercially available *N. caninum* iscom ELISA kit (Svanova Biotech AB, Sweden). The kit was used according to the manufacturer's instructions. Briefly, 100 microlitres of pre-diluted serum sample added as first antibody and the plate incubated at 37 °C on shaker for 1 hour. The wells were washed three times with PBS Tween Buffer and 100 microlitres of HRP conjugate added to each well and incubated for one hour at 37 °C. The plate was washed again and 100 microlitres of substrate solution added and incubated at room temperature for 10 minutes. Then 50 microlitres of stop solution were added to stop the reaction and the plates were read in an ELISA microplate reader (Anthos 2020, Austria) at a wavelength of 450 nm. The optical density (OD) of the ELISA was read on an automatic plate reader and the Percent Positivity values (PP) of the test samples were calculated by the following formula:

$$PP = \frac{\text{Mean OD value (sample or Negative Control)} \times 100}{\text{Mean OD value Positive Control}}$$

The results were expressed as the percent positivity (PP) of the high positive control sera. The manufacturer's current recommendations for the interpretation of the test are that a test result of below 20 PP indicates a negative result, and a test result of above or equal to 20 PP indicates a positive result.

Statistical analysis

Descriptive statistics with 95% confidence interval (CI) was used to estimate the prevalence of infection and a chi-square test was used to analyze associations between infection by *N. caninum* and other factors studied in the present study. For statistical analysis, the SPSS 12 computer program was used and P<0.05 was considered to be significant.

Table 1: Seroprevalence of *Neospora caninum* in relation to age (95% CI)

Age	The number of animals tested	No. of positives	Seroprevalence (%)
<18 months	29	6	20.6%
≥18 months	71	20	28.1%

Table 2: Comparison of *Neospora caninum* antibodies in relation to sex (95% CI)

Sex	The number of animals tested	No. of positives	Seroprevalence (%)
Bull	9	2	22.2%
Cow	91	24	26.3%

Results

Results obtained from the sera using ELISA are given in Tables 1 and 2. The results were expressed as the percent positivity (PP) of the high positive control sera. Antibodies to *N. caninum* were found in 26 of the 100 (26.0%; 95% CI: 17.8-35.7) sera based on ELISA results. Among the 29 sera in the cattle <18 month age group, 6 (20.7%; 95% CI: 8.0-39.7) were seropositive, whereas among the 71 sera above 18 months old, 20 (28.2%; 95% CI: 18.1-40.0) were seropositive (Table 1). Among the 9 bulls, 2 (22.2%; 2.8-60.0) were seropositive whereas of the 91 cows, 24 (26.4%; 95% CI: 17.7-36.7) were seropositive (Table 2). There was no statistically significant relationship between seroprevalence of sex and age groups ($P>0.05$)

Discussion

Our study showed more than one fourth of samples were positive for *N. caninum*, which can cause abortion in cattle leading to economic loss in the cattle industry in this region. In this study there was no significant difference in seroprevalence between the different age groups.

Akca et al. (2005) reported that 8.2% of Simmental tested cows were positive in Kars province, Turkey (21). Sevgili et al. (2005) found antibodies to *N. caninum* in 23 of the 305 (7.5%) cow sera based on ELISA test results in the province of Sanliurfa, Turkey (22). With regard to seropositivity, no significant difference was observed in origin, animal breed, and age ($P>0.05$).

The presence of antibodies against *N. caninum* in cows only indicates exposure to the parasite. Wouda et al. (1998) and Sadrebazzaz et al. (20) reported for most herds that the seroprevalence levels were equal across all age groups (23). The relationship between age and seroprevalence in bovine neosporosis is speculative. Jensen et al. (1999) suggested that seroprevalence increases with age (24). In contrast, Sanderson et al. (2000) reported that cows below 3 years of age had higher CI-ELISA inhibition percentage values than cows above 6 years of age (25). They also suggested that infected cows can infect fetuses, and if these calves have not been reinfected, antibody titers decline over time, resulting in an apparent decrease in seroprevalence with cow age. In our previous study (3) in Kerman province, antibodies to *N. caninum* were found in 36 of the 285 (12.6%) sera based on ELISA test results. Data showed that no significant difference regarding origin, sex and age ($P>0.05$).

Heidari et al (2012) reported that the seroprevalence of *N. caninum* infection in the native cattle of Kurdistan province of Iran is 7.80% (26). Their results showed that there was no significant difference in the prevalence rate of abortion between seropositive and non-seropositive cattle ($p = 0.588$). These findings may be due to that native breeds are genetically resistant to neosporosis. In investigation in Brazil on the infection of *N. caninum* in different cattle breeds (Zebu, Holstein, crossbreed Zebu/Holstein) there was close association between cattle breeds and the frequency of infection by *N. caninum* (10). The result of previous study

indicated that breed, which is inherent factor to host, could be considered of high relevance for the distribution and frequency of infection by *N. caninum*. The authors of that study suggested that genetic distance between Holstein and Zebu breeds possibly leads to the significant difference. These results were similar to finding of a study performed in Ontario which showed that there was a genetic susceptibility to infection by *N. caninum* in Holstein cattle (27).

Due to the lack of information about the prevalence of infection in the definitive host, the dog, in Iran, it is not possible to know which method of transmission (horizontal or vertical) is the main route of infection. However, further studies on the epidemiological evidence for a relationship between *N. caninum* infection in dogs and cattle and the relationship between abortion in cows and infection with *N. caninum* in Iran are required.

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SEROPREVALENCA *Neospora caninum* PRI GOVEDU V SEVERNEM IRANU

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Povzetek: *Neospora caninum* je po vsem svetu razširjen patogen, ki povzroča zvrge pri govedu, kar ima za posledico v velike gospodarske izgube v govedoreji. Cilj naše raziskave je bil ugotoviti prekuženost s protitelesi proti *N. caninum* pri govedu v mestu Neishabour (severovzhodni Iran). Od septembra 2012 do oktobra 2013 je bilo analiziranih skupno 100 vzorcev seruma za protitelesa proti antigenu *N. caninum* z uporabo komercialno dostopnega kita *N. caninum* ISCOMELISA. Rezultati testa ELISA so pokazali protitelesa proti *N. caninum* v 26 od 100 vzorcev (26%) serumov. V zvezi s seropozitivnostjo niso opazili pomembnih razlik glede pripadnosti, spola in starosti ($p > 0,05$). Rezultati so pokazali, da je *N. caninum* dokaj razširjena pri govedu v severovzhodnem delu Irana in da je ovrednotenje možnosti okužbe lahko koristno pri pripravi kontrolnih programov.

Ključne besede: seroprevalenca; *Neospora caninum*; Neishabour; govedo; Iran